Carbon 140 (2018) 352–361

Contents lists available at ScienceDirect

Carbon

journal homepage: www.elsevier.com/locate/carbon

Effects of the size and oxidation of graphene oxide on crop quality and specific molecular pathways

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ARTICLE INFO

Article history:
Received 11 July 2018
Received in revised form
28 August 2018
Accepted 29 August 2018
Available online 31 August 2018

ABSTRACT

Applications of graphene-based nanomaterials in agriculture have attracted much attention, but their potential risks to crop quality and food safety are largely unknown. The present study found that graphene oxide (GO), GO quantum dots (GOQDs) and reduced GO (rGO) translocated from wheat stems to grains and formed large nanomaterial aggregates. The nanomaterials also reduced the globulin, prolamin, amylose and amylopectin contents by 8–28%, 11–25%, 5–34%, and 23–37%, respectively, decreased the levels of mineral elements and upregulated the soluble sugar content by 19–36% in wheat grains, while rGO downregulated the levels of proteins with nutrient reservoir activity to a greater extent than GO. The downregulation of alpha-amylase inhibitor was responsible for the observed decrease in starch content in grains. The decrease in the mineral element contents obtained with rGO and GOQD was greater than that observed with GO, and this effect was linked to the upregulation of calmodulin mediated by ABC transporters. GOQD and rGO changed the proteomic and metabolomic profiles more strongly than GO, suggesting that graphene materials with a small size and a low oxidation content are clearly more detrimental to grain quality. The above results provide an important basis for further nanomaterial design and agricultural applications.

1. Introduction

The application of graphene-based nanomaterials (GBNs) has attracted much attention in agricultural management to solve food crises and improve food production (e.g., as nanobiosensors, carriers of fertilizers, pesticide delivery vectors and plant growth regulators) [1–5]. However, the adverse effects of nanomaterials on agricultural applications might be harmful to food quality [6–10]. The phytotoxicity of GBNs has been widely studied in the laboratory, i.e., in studies of the inhibition of growth and photosynthesis in cabbage, red spinach, lettuce and wheat [11,12]. In general, the results of laboratory and wheat field studies are not completely comparable because the complex factors in real environments cannot be completely simulated in the laboratory [13]. However, the effect of GBNs on corn safety is largely unknown.

Based on the size and surface chemistry of these nanomaterials, GBNs typically include graphene, graphene oxide (GO), GO quantum dots (GOQD) and reduced GO (rGO) [14], and the size and surface chemistry of these nanomaterials affect their nanosafety. In addition, interlaboratory comparisons have led to many arguments due to differences in experimental conditions and nanomaterial properties [15]. Over the past ten years, obvious progress in the phytotoxicity of nanoparticles has been achieved, and the specific improvements include advances in oxidative stress, root elongation, and biomass production in plant seedlings [16]. Crops are the main source of human food, and crop quality is essential to food safety and human health; however, the effects of the size and surface chemistry of GBNs on crop quality (e.g., the content of nutritional sugars, proteins and mineral elements) remain largely unknown. Understanding the effects of the size and surface chemistry of nanomaterials on crop quality in wheat fields is critical for the design of safe nanomaterials and the scientific assessment of the risks associated with the application of nanomaterials.

In addition, the study of only the few proteins and metabolites that function as traditional biological end points might not provide all the biological information that is important for nanosafety and

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https://doi.org/10.1016/j.carbon.2018.08.063
0008-6223/

might fail to provide comprehensive information to explain the mechanisms of biological responses [17,18]. Untargeted omics could solve this problem [19]. In the present work, proteomics and metabolomics were integrated with various biological end points of crop quality, such as protein, starch, soluble sugar and mineral element contents, to reveal the specific mechanisms through which GBNs affect crop quality in a wheat field. Wheat is the most important food crop in the world and accounts for approximately 30% of the total cereal production worldwide [20,21]. Studying the size and oxidation level of nanomaterials mediating wheat quality in a wheat field and the specific related molecular pathways using associated omics methods will provide a deeper scientific understanding of the potential risks of emerging nanomaterials.

2. Experimental

2.1. Nanoparticle characterization

GO (production number, XF002-1), GOQD (production number, XF042) and rGO (production number, XF032) were obtained from the Nanjing XFNANO Materials Tech Co., Ltd., China. The methods used for nanoparticle characterization were described in detail in our recent studies [22,23]. Briefly, the nanoparticle morphology was examined by field emission transmission electron microscopy (TEM, JEM-2010 FEF, JEOL, Japan) and atomic force microscopy (AFM, NanoScope IV, Veeco, USA). Hydrodynamic diameters (Hds) were obtained using a Zetasizer Nano ZS90 instrument (Malvern, UK). The Raman spectra of GBNs were analyzed using a Raman spectrometer (DXR Microscope, Thermo Scientific, USA) with an excitation wavelength of 532 nm from a diode-pumped solid-state (DPSS) laser. The proportions of O in GBNs were analyzed using an automatic elemental analyzer (Euro EA 3000, Leeman, USA).

2.2. Study of wheat field performance

Wheat is an important food source and is eaten by more than one billion people [20,21]. Tianjin (39.1° N, 117.2°E), located in northern China, has a subhumid, warm, temperate, continental monsoon climate and is very suitable for the growth of winter wheat. Tianjin has four distinct seasons and abundant sunshine. The annual average temperature is 11.3–12.8 °C, and the average annual rainfall is 571 mm. The average temperatures in January and July are −5 ~ −3 °C and 26–27 °C, respectively. To investigate the effects of nanomaterials on food safety, a wheat field was selected. A specific wheat field was selected in Nanyi Village, Tianjin, China. The pH in the soil was approximately 7.9, and the soil contained 1.02% clay (<2 μm), 40.87% silt (2–20 μm) and 58.11% fine sand (20–200 μm). The contents of total organic carbon and total nitrogen in the soil were 69 mg/100 g dry soil and 1.01 g/100 g dry soil, respectively.

In October 2016, wheat seeds (Cangmai 026, Cangzhou Academy of Agricultural and Forestry Sciences, Hebei, China) were evenly planted in a wheat field using a wheat planter and subjected to regular irrigation and fertilization management strategies. The grain filling stage is crucial for nutrient (e.g., starch and protein) accumulation, sensitive to extrinsic stress (such as diseases and insects), and an important stage for nanomaterial applications in agriculture [24,25]. Herein, wheat was exposed to GBNs at the grain filling stage in the middle of May 2017. In the early part of the filling stage, farmland that was 3-m wide and 15-m long was selected as the test wheat field, where well-developed wheat presented a consistent shape.

Although the environmental concentrations of GBNs are unclear, the concentrations of carbon-based nanomaterials in direct agricultural applications reached parts per million (ppm) levels [26]. When crop plants were exposed to carbon-based nanomaterials at the level of mg/L (ppm), the uptake concentrations in crop plant sheath/stem reached the level of mg/kg, whereas the predicted concentrations in water or soil reached parts per billion (ppb) [27]. In addition to direct soil exposure, stem injection represents an exposure pathway for the effective application of nanoenabled fertilizers and pesticides [26,28,29]. To evaluate the effects of the direct agricultural application and unintentional contamination of carbon-based nanomaterials on crop quality, plant stems were injected once with GBNs at concentrations of 0.5 μg/kg, 5 μg/kg and 50 μg/kg fresh weight in the field study. The stem injection method was adopted from the spore injection method to test the phytotoxicity [29,30]. Specifically, GBNs were injected into the medullary cavity of stems (i.e., injection depth). The injected position was located approximately 10 cm away from the wheat ears (first internode below the wheat ears). The injection was performed using a micro-injector (5-μL injection needle with a beveled tip, W-131, Shanghai Guangzheng Medical Equipment Co., Ltd., China). An equal volume of deionized water was injected in the control. As described in a previous study, the GBMs also exhibited good dispersion in natural water with low concentrations of ions and natural organic matters [31].

Two campaigns of nanoparticle injection were performed: the first was performed on 18 May 2017, and the second one was performed on 25 May 2017. At the wheat maturation stage (8 June 2017), the grains were collected from the wheat ears, stored at −80 °C, and analyzed soon thereafter.

2.3. Nanoparticle uptake

The collected grains were washed with deionized water and cross-sectioned at the middle with a sharp stainless-steel knife. The cross-sections were placed on glass slides. To identify the uptake of GBNs, the typical D and G bands were analyzed using a Raman spectrometer (DXR Microscope, Thermo Scientific, USA) with an excitation wavelength of 532 nm from a DPSS laser. A point-scan confocal Raman system was utilized to construct Raman maps of D and G bands of the GBNs and thus analyze the biotransformation of GBNs in grains.

2.4. Assay of extractable protein fractions

The extractable protein fractions (albumin, globulin, prolamin and glutelin fractions) of wheat grains were collected according to a previously published method with minor modifications [32,33]. The wheat grains (200 mg) were rinsed repeatedly with distilled water and ground with a mortar. The albumin was extracted with 1 mL of distilled water with gentle shaking (150 r/min) for 2 h at 4 °C and then centrifuged at 15, 000 g for 15 min. This extraction was repeated three times. After water extraction, globulin, prolamin and glutelin were extracted with 1 mL of 0.5 M NaCl, 1 mL of 70% ethanol and 1 mL of 0.1 M NaOH, respectively. The processes of shaking and centrifugation were executed as described above, and the proteins in the supernatant were collected. The extracted proteins were quantified using bicinchoninic acid (BCA) assay kits (A045-4, Nanjing Jiancheng Bioengineering Institute, China) according to the manufacturer's instructions.

2.5. Analysis of the secondary structure of proteins

The wheat grains were rinsed with deionized water and then lyophilized. Subsequently, the lyophilized wheat grains were ground, and 1 mg of powder was mixed with 200 mg of dried KBr powder. The samples were analyzed using a Fourier transform infrared (FT-IR) spectrometer (Bruker Tensor 27, Germany). Grain